Prometaphase Chromosome Preparation from Mouse Spleen (C57Bl/6)

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National Institutes of Health

Reagents

Acetic acid, glacial
Antibiotic-Antimycotic 100x
10,000 U/ml Penicillin G sodium, 10,000 µg/ml streptomycin sulfate, 25 µg/ml amphotericin B
Invitrogen Corp., Cat. 15240-013
Bromodeoxyaridine (BrdU)
Sigma, Cat. B9285
Colcemid, KaryoMAX Colcemid Solution, 10 µg/ml
Invitrogen Corp., Cat. 15210-016
Concanavalin A (5 µg/µl)
Sigma, Cat. C-5275
Fetal Bovine Serum (FBS) heat inactivated
Invitrogen Corp., 16140-022
L-Glutamine-200 mM, 100x
Invitrogen Corp., 25030-016
Homogenizer
Thomas Scientific, Cat. 3431D7
Lipopolysaccharides (LPS) 5mg
Sigma, Cat. L-2637
Methyl alcohol, anhydrous
Mallinckrodt, Cat. 3016
Methotrexate (MTX), 500 mg
Sigma, Cat. M 8407
Potassium chloride (KCl)
RPMI Medium 1640
Invitrogen Corp., Cat. 21870-050

Preparation

Reagents

	Amount
Concanavalin A	
Concanavalin A	5 mg
Sterile water	1 ml

For a stock solution of 5μg/μl

RPMI 1640 Complete Medium

Components	**Amount**
RPMI Medium 1640	440 ml
Antibiotic-Antimycotic, 100X	5 ml
L-Glutamine-200 mM, 100X	5 ml
Fetal Bovine Serum (FBS)	50 ml

Fixative

Prepare fresh: methanol/acetic acid 3:1, volume:volume

Hypotonic Solution: 0.075M KCl

KCl	5.6 g
Distilled water	1000 ml

Lipopolysaccharides (LPS), stock solution

Lipopolysaccharides (LPS)	25 mg
Sterile water	1 ml

Use 1:1000 dilution for a final concentration of 25 μg/ml of culture

MTX stock

Make an initial stock of 10^{-3} M in H_2O and then dilute to 10^{-5} M
Prepare fresh with each use.

BrdU stock

1 mg/ml in distilled water
Prepare fresh with each use.

Procedure

1. Prepare tissue culture flasks. To one T75 flask, add:

Components	Amount
Prepared media	20 ml
Concanavalin A (5μg/μl)	30 μl
Lipopolysaccharides (LPS)	25 μl

1. Isolate spleen from mouse. Transport in sterile, unsupplemented RPMI 1640.

3. Place three spleens into a homogenizer with 3 ml of plain RPMI media. Grind well.

4. Transfer 0.5 ml of cell suspension to each T75 flask.

5. Incubate at 37°C for 24 hr. After 24 hr add 200 μl of MTX stock (10^{-5}M) to 20

ml of culture (MTX final concentration of 10^{-7}M); mix well and incubate an additional 17 h.

6. After 17 hr centrifuge the content of the flasks, remove the supernatant, and wash the pellet twice with plain media.

7. After the second wash resuspend the pellet in 20 ml of RPMI 1640 10% BSA and transfer to a T75 flask.

8. Add 500 µl of the BrdU stock (1mg/ml) to a final concentration of 25 μg/ml (minimize light exposure).

9. Incubate for 5 hr 30 min at 37°C.

10. For the last 10 min of the incubation add 20 µl of Colcemid stock (10 μg/ml) to a final concentration of 0.06 μg/ml .

11. Centrifuge cultures for 10 min.

12. Transfer to 50 ml centrifuge tubes and centrifuge at 1,000 rpm for 10 min.

13. Remove supernatant.

14. Gently add 10 ml 0.075M KCl (prewarmed to 37°C) to each tube and resuspend pellet.

15. Incubate tubes at 37°C for 15 min.

16. Following incubation, add a few drops of freshly prepared fixative.

17. Centrifuge at 1,200 rpm for 10 min.

18. Remove supernatant.

19. Wash pellet with freshly prepared fixative, at least 3 times.

20. Store pellet under fixative at -20°C until ready to prepare slides.